SUPPORTING INFORMATION

Magnetic separation of elastin-like polypeptide receptors for enrichment of cellular and molecular targets

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Experimental details

Materials. Unless stated otherwise, all chemicals and reagents were purchased from Sigma. Restriction enzymes and *E. coli* BL21(*DE3*) cells were purchased from New England Biolabs. Antibodies were bought from Thermo Scientific. The pET28a plasmids encoding the Coh (Addgene plasmid #58709) and GFP-Doc (Addgene #58708) were the same as from Otten et al.[1] The pET28a plasmid encoding the iLOV-Doc was a gift from Markus Jobst and Wolfgang Ott (Ludwig-Maximilians-Universität, Germany). The yeast display vector, pYD1 was a gift from Dane Wittrup (Addgene plasmid #73447).[2] Fresh frozen plasma was obtained from the Basel University Hospital (Blutspendezentrum SRK beider Basel).

Synthesis of pNIPAm and pNIPAm-mNPs

pNIPAm. An amount of 2g NIPAm was dissolved in 5 ml dioxane. After that, 145 mg 2-(Dodecylthiocarbonothioylthio)-2-methylpropionic (DDMAT) and 6 mg 4,4′-Azobis(4-cyanovaleric acid) was added. The mixture was purged with argon gas for 20 min and reacted at 60 ^{o}C overnight. The mixture was precipitated in a 3 fold-excess volume of n-pentane and dried at 80 ^{o}C overnight. The precipitate was re-dissolved in MQ water and dialysed against MQ water. The final product was lyophilized and stored at 4 ^{o}C until further use.

pNIPAm-mNPs. To 1 mole of pNIPAm polymer, 50 mole tetraglyme was added and the mixture was heated at 100 ^{o}C for 30 min or until the polymer was completely dissolved. After that, iron(0)-pentacarbonyl filtered through a 0.45 µm membrane was added to the mixture at a ratio of 1:250 (v/v). The reaction was carried out at 100 ^{o}C while stirring for 10 min, and then the temperature was raised to 180 ^{o}C for 5 hrs. The reaction mix was next cooled to room temperature and precipitated into cold n-hexane. The precipitate was dried under vacuum, re-dissolved in MQ water and dialysed against MQ water at least 3 times to remove residual solvent. The final product was lyophilized and stored at -20 ^{o}C until further use.

Synthesis of ELP-Coh, iLOV-Doc and Coh-iLOV

Molecular cloning

The ELP gene with the guest residue composition of $M_1V_7E_2$ was synthesized by the recursive directional ligation method.[3] Briefly, a short ELP gene encoding the $M_1V_7E_2$ sequence was designed with four flanking restriction sites: *Bam*HI, *Pfl*MI, *Bgl*I and *Xba*I; and was synthesized and cloned into the pMA vector by GeneArt (Thermo Scientific). The *Pfl*MI and *Bgl*I sites were designed to have different recognition sites that when cut generated complementary sticky ends. In one digest/ligation cycle, the plasmid was cut with either *Pfl*MI only or both *Pfl*MI and *Bgl*I, which generated the linear vector or the insert, respectively. The insert was then re-ligated into the linear vector, resulting two ELP segment duplicates connected by a VPGVG pentapeptide without any restriction site in between. The cloning cycle was then repeated with the newly obtained vector, thus the size of the ELP gene was doubled after each cycle. The ELPs with desired lengths were then ligated to the expression vector pET28a using *Bam*HI and *Xba*I sites, yielding the pET28a-ELP constructs. Finally, the Coh gene (amplified from the pET28a-Coh-iLOV) was ligated into these plasmids using *Bgl*I and *Xho*I sites. On the other hand, the His_6-iLOV gene (amplified from the pET28a-Coh-iLOV) was ligated into the pET28a-GFP-Doc plasmid (GFP removed) using *Nhe*I and *Xba*I sites for bacterial expression of the iLOV-Doc fusion protein. The pET28a-Coh-iLOV was used for expression of the Coh-iLOV for negative control. All plasmids were verified by sequencing before expression.

Protein expression and purification

All proteins were expressed in *E. coli* BL21(*DE3*) cells. Cells were grown in 5 mL LB supplemented with 50 µg/mL kanamycin at 37 ^{o}C overnight with shaking. On the next day, 4 mL

of this preculture was inoculated into 400 mL LB supplemented with 50 µg/mL kanamycin. The culture was grown at 37 ^{o}C with shaking until the OD_{600} reached 0.5. After that, protein expression was induced with 1 mM IPTG. The expression was performed at 25 ^{o}C for 6 hrs with shaking. The cells were then harvested by centrifugation at 4000g for 10 min and re-suspended in 20 mL lysis buffer (50 mM Tris, 50 mM NaCl pH 8.0 containing 1 mg lysozyme and 20 Units of *DNase*I) and incubated on ice for 15 min before sonication (35% amplitude, 2 second pulse on, 2 second pulse off) for 10 min. Cell lysate was then centrifuged at 18000g, 4 ^{o}C for 30 min. For ELP-Coh, the clear supernatant was subjected to Inverse Transition Cycling (ITC) with three rounds. In the first ITC round, 5M NaCl was added to the supernatant to trigger the phase transition of the ELP at room temperature (RT) by which the cell lysate became turbid due to ELP aggregation and precipitation. The mixture was then centrifuged at 14000g at RT for 10 min and the supernatant was discarded. The pellet was re-suspended in 1 mL cold PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4), followed by centrifugation at 14000g at 4 ^{o}C for 10 min to remove denatured, insoluble contaminants. The clear supernatant then underwent two more ITC rounds with 3M NaCl and the ELPs were obtained in final resuspension with 0.5 mL cold PBS. On the other hand, the His_6-tagged iLOV-Doc and Coh-iLOV were purified on a His-Trap FF column using an AKTA system. The purified proteins were analysed on 12% SDS-PAGE gel and the protein concentration was determined by using the BCA assay. Binding of the ELP-Coh to the iLOV-Doc was verified by SEC using a Superose 12 10/300 GL column (GE Healthcare) with PBS as running buffer and a 0.5 $ml.min^{-1}$ flowrate.

Preparation of yeast cells displaying GFP-Doc or iLOV-Doc

GFP-Doc and iLOV-Doc genes (amplified from the pET28a-GFP-Doc or pET28a-iLOV-Doc, respectively) were cloned into the pYD1 vector using the *Bam*HI and *Xho*I sites. This allows for expression of the Aga2p-GFP(iLOV)-Doc fusion proteins, resulting the display of these proteins on yeast cell-wall via Aga1p-Aga2p interaction.[4] The vectors were transformed to EBY100 yeast cells[4] using a routine lithium acetate protocol.[5] Positive clones were selected on SD agar plates containing 2% (w/v) glucose and lacking tryptophan (-TRP). Resulting colonies were cultivated in liquid SD -TRP medium with 2% (w/v) glucose for 24 hrs at 30 °C and continuous shaking at 200 rpm. Protein expression and display were then induced by transferring the culture to a fresh liquid medium lacking tryptophan containing 0.2% (w/v) glucose and 1.8% (w/v) galactose, and shaking for 40 hours at 20 °C. After that, the cells were spun down, resuspended and diluted in sterile PBS to a desired density, or kept at -20 °C if not used immediately. The display efficiency was then determined by flow cytometry as described later.

Thermoresponsive characterization of the mNPs and ELP-Coh

pH responsive characterization was performed using a cloud-point assay. The mNPs or ELP-Coh was dissolved at a final concentration of 0.5 mg/mL in different buffers including: 20 mM acetate buffer pH 4.5 or 5.5; 20 mM sodium phosphate buffer pH 6.5; PBS pH 7.4; 20 mM HEPES containing 137 mM NaCl pH 8.5. The solutions were heated from 15 ^{o}C to 85 ^{o}C with a rate of 0.5 ^{o}C/min using an Evolution™ 260 Bio UV-Visible Spectrophotometer (Thermo Scientific). The absorbance at 700 nm (for mNPs) or 350 nm (for ELP-Coh) was plotted as a function of temperature (Figure 1B and C). As temperature increases to a certain value, the mNPs or ELP-Coh start to collapse and the absorbance increases. The transition temperature of the mNPs or ELP-Coh solution was determined as the temperature at which the absorbance is equal to 50% of the maximum value. Ionic strength responsive characterization was performed similarly. The mNPs or ELP-Coh was dissolved at a final concentration of 0.5 mg/mL in PBS supplemented with different NaCl concentrations (137 mM, 0.5 M, 1M, 1.5M, 2M) and was subjected to the cloud-point assay as described above.

Cross-aggregation of the mNPs and ELP-Coh

The mNPs and ELP-Coh were dissolved at a final concentration of 1.5 and 0.5 mg/ml, respectively, in 1 ml of collapsing buffer (20 mM acetate buffer pH 4.5 containing 0.5M NaCl) in a semi-micro cuvette. The mixture was incubated at 40 ^{o}C for 1 min to trigger the phase-transition of both mNPs and ELP-Coh. The cuvette was then placed on a Neodymium super magnet for 1 min to sediment the mNPs-ELP-Coh complex. The supernatant was removed (sampled as 'Sup1') and the sediment was washed with 1 ml of collapsing buffer. The phase transition and sediment was repeated and the supernatant was decanted (sampled as 'Wash'). After that, 1 ml of releasing buffer (PBS pH 7.4 containing 0.5M NaCl) was added to re-solubilize the pNIPAm-mNPs/ELP-Coh complex. Re-solubilization could be enhanced by incubating the cuvette at 4 ^{o}C for 5 min. Finally, the mixture was incubated at 40 ^{o}C for 1 min to trigger the phase transition of the mNPs only, which was then sediment by the super magnet. The supernatant was removed (sampled as 'Sup2'). Protein contents of 'Sup1', 'Wash' and 'Sup2' fractions were determined by BCA assay. The experiment was performed in triplicates.

Capture of free iLOV-Doc

The ELP-Coh and iLOV-Doc (or Coh-iLOV as non-binding protein) was mixed in 80 µl TBS-Ca buffer (25 mM Tris, 72 mM NaCl, 1 mM $CaCl_2$) in a 96-well microtiter plate for 5 min at RT. After that, mNPs were added and the volume was adjusted to 200 µl with collapsing buffer. The final concentrations of the iLOV-Doc, ELP-Coh and mNPs were 5 µM (0.13 mg/ml), 9 µM (0.5 mg/ml) and 1.5 mg/ml, respectively. The plate was incubated at 40 ^{o}C for 1 min to trigger the phase-transition of both mNPs and ELP-Coh and was then placed on a super magnet plate (V&P Scientific, VP771RM-1) for 1 min to sediment the pNIPAm-mNPs/ELP-Coh complex. The supernatant was removed (sampled as 'Sup1') and the sediment was washed with 200 µl of collapsing buffer. The phase transition and sediment was repeated and the supernatant was decanted (sampled as 'Wash'). After that, 200 µl of releasing buffer was added to re-solubilize the pNIPAm-mNPs/ELP-Coh complex. Finally, the mixture was incubated at 40 ^{o}C for 1 min to trigger the phase transition of the mNPs only, which was then separated by the magnet plate. The supernatant was removed (sampled as 'Sup2'). Samples containing only iLOV-Doc (5 µM) in PBS were used as positive control. Samples with ELP-Coh and iLOV-Doc and without mNPs were used as negative control. Protein contents of 'Sup1', 'Wash' and 'Sup2' fractions were analyzed for fluorescence using a SAFIREII microplate-reader (excitation/emission wavelength: 450/495 nm).

In order to use the pNIPAm-mNPs/ELP-Coh system for isolation of low-concentration protein in a mixture with other proteins, a starting solution containing 1.3 µg/ml (0.05 µM) of iLOV-Doc with or without 20 µg/ml (0.3 µM) BSA was mixed with ELP-Coh and mNPs as described above in five wells. At the final step, 200 µl of releasing buffer was added to the first well, resuspended and transferred to the next well and so on. Fluorescence intensity of the initial and enriched solutions was determined and normalized against the pure buffer and protein content was analyzed on 12% SDS-PAGE.

In order to test the capability of the system for capturing target proteins in biological samples, iLOV-Doc was spiked at different concentrations into 10% human blood plasma in TBS-Ca buffer. Separation was performed similarly to the procedure in clean buffer except for that 5 mM $CaCl_2$ was added for ELP-Coh binding and 2x collapsing buffer was used for pNIPAm-mNPs/ELP-Coh co-aggregation. Fluorescence intensity of the initial and recovered solutions was determined and normalized against the pure buffer and pure buffer supplemented with 10% plasma. Protein content was analyzed on 12% SDS-PAGE.

All experiments were performed in triplicates.

Capture of yeast cells displaying GFP-Doc or iLOV-Doc

The ELP-Coh (100 µg) was mixed with 5 x 10^5 cells (i.e. ~6.25 x 10^6 cells/mL) with varied percentages of GFP-positive cells) in 80 µl of TBS-Ca buffer (or TBS-Ca buffer supplemented with 5 mM $CaCl_2$ and 10% human plasma) in a 96-well microtiter plate for 5 min at RT. After that, mNPs was added and the volume was adjusted to 200 µl with 1x collapsing buffer (or 2x collapsing buffer if working with plasma sample). The final concentration of the ELP-Coh and mNPs was 0.5 mg/ml and 1.5 mg/ml, respectively. The mixture was incubated at 40 ^oC for 1 min to trigger the phase-transition of both mNPs and ELP-Coh. The plate was then placed on a super magnet plate-holder for 1 min to sediment the pNIPAm-mNPs/ELP-Coh complex. The supernatant was removed (sampled as 'Sup1') and the sediment was washed with 200 µl of collapsing buffer. The phase transition and sedimentation were repeated and the supernatant was decanted (sampled as 'Wash'). After that, 200 µl of releasing buffer were added to re-solubilize the mNPs-ELP-Coh complex. Re-solubilization could be enhanced by incubating the cuvette at 4 ^oC for 5 min. Finally, the mixture was incubated at 40 ^oC for 1 min to trigger the phase transition of the mNPs only, which was then sedimented by the super magnet. The supernatant was removed (sampled as 'Sup2'). The 'Sup1', 'Wash' and 'Sup2' fractions were diluted to 500 µl with PBS for analysis by flow cytometry as described below. All experiments were performed in triplicates.

Flow cytometry analysis

Quality control of cell display efficiency. All the yeast cells were analyzed with the Attune NxT (Thermo Fisher Scientific) flow cytometer equipped with a 488 nm and a 561 nm laser for detecting green and red fluorescence, respectively. The OD_{600} of the cell resuspension (in PBS) was measured to determine cell density (1 OD is equivalent to 10^7 cells in 1 ml). A fraction of around 2 x 10^6 cells was sampled. The cells were spun down at 14000g at 4 °C for 30 sec, then washed with 1 ml ice-cold PBS containing 0.1% BSA. The cell suspension was then divided into two fractions and spun down at 14000g at 4 °C for 30 sec; one fraction (used as negative control) was then resuspended in 50 µl ice-cold PBS containing 0.1% BSA and another one was resuspended in the same buffer supplemented with 1:1000 diluted mouse anti-Xpress tag antibody. The cells were incubated at room temperature for 30 min. After that, the cells were spun down at 14000g at 4 °C for 30 sec, washed with 1 ml ice-cold PBS containing 0.1% BSA and resuspensded in 50 µl ice-cold PBS containing 0.1% BSA and 1:1000 diluted goat anti-mouse IgG antibody conjugated with Alexa555 dye. The samples were incubated at room temperature for 20 min. After that, the cells were spun down at 14000g at 4 °C for 30 sec, washed with 1 ml ice-cold PBS containing 0.1% BSA and resuspended in 500 µl ice-cold PBS containing 0.1% BSA. For flow cytometry, 100 µl of the sample was analyzed at 50 $\mu l.min^{-1}$ flow-rate. Wild-type yeast cells and the negative control fraction were used to set the gate for fluorescence analysis.

Cell capture analysis. The cell population without protein display was used to set the gate for the analysis. A volume of 100 µl of the sample was analyzed at 50 $\mu l.min^{-1}$ flow-rate. The same setting was applied to analyze the 'Sup1', 'Wash' and 'Sup2' fractions of all the samples subjected to magnetic separation in order to determine the number of GFP-positive cells. The cell capture efficiency was defined as the percentage of captured positive cells with respect to the total number of positive cells in the original sample. The enrichment factor was defined as the ratio between the percentage of positive cells in the recovered sample to that in the corresponding starting sample.

Determination of detection limit. Fluorescence signal (for detection of iLOV-Doc) or positive cell count (for detection of yeast cells displaying GFP-Doc) as functions of initial spiked iLOV-Doc

concentration or GFP-Doc displaying cells were plotted and linear regression was performed. From the obtained regression model (equation), the detection limit (DL) can be then determined by using the formula: DL = 3.3 x (standard error of intercept) / slope.[6]

References

(1) Otten, M., Ott, W., Jobst, M. A., Milles, L., Verdorfer, T., Pippig, D., Nash, M. A., Gaub, H. E. *Nat. Methods* **2014** *11*, 1127–1130.
(2) Kieke, M. C.; Cho, B. K.; Boder, E. T.; Kranz, D. M.; Wittrup, K. D. *Protein Eng.* **1997**, *10*, 1303–1310.
(3) Meyer and Ashutosh, D. *Biomacromolecules* **2002**, *3*, 357–367.
(4) Boder, E. T.; Wittrup, K. D. *Nat. Biotechnol.* **1997**, *15*, 553–557.
(5) Gietz, R. D.; Woods, R. A. *Methods Enzymol.* **2002**, *350*, 87–96.
(6) Swartz, M.E. and Krull, I.S., *Handbook of analytical validation. Chapter 4: Method validation basics*. 2012: CRC Press Taylor & Francis Group.

Amino acid sequences

ELP-Coh(s) (blue: ELP, yellow: Cohesin)

$(M_1V_7E_2)_9$-Coh

MGTDSLEFIASKLAWGSGHGVGVPGMGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGEG
VPGEGVPGVGVPGMGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGEGVPGEGVPGVGV
PGMGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGEGVPGEGVPGVGVPGMGVPGVGVP
GVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGEGVPGEGVPGVGVPGMGVPGVGVPGVGVPGVGVPG
VGVPGVGVPGVGVPGVGVPGEGVPGEGVPGVGVPGMGVPGVGVPGVGVPGVGVPGVGVPGVGVPGV
GVPGVGVPGEGVPGEGVPGVGVPGMGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGEG
VPGEGVPGVGVPGMGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGEGVPGEGVPGVGV
PGMGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGEGVPGEGVPGWGSDGVVVEIGKVT
GSVGTTVEIPVYFRGVPSKGIANCDFVFRYDPNVLEIIGIDPGDIIVDPNPTKSFDTAIYPDRKIIVFLFAEDSG
TGAYAITKDGVFAKIRATVKSSAPGYITFDEVGGFADNDLVEQKVSFIDGGVNVGNAT

$(M_1V_7E_2)_{12}$-Coh

MGTDSLEFIASKLAWGSGHGVGVPGMGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGEG
VPGEGVPGVGVPGMGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGEGVPGEGVPGVGV
PGMGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGEGVPGEGVPGVGVPGMGVPGVGVP
GVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGEGVPGEGVPGVGVPGMGVPGVGVP
VGVPGVGVPGVGVPGVGVPGEGVPGEGVPGVGVPGMGVPGVGVPGVGVPGVGVPGVGVPGVGVPGV
GVPGVGVPGEGVPGEGVPGVGVPGMGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGEG
VPGEGVPGVGVPGMGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGEGVPGEGVPGVGV
PGMGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGEGVPGEGVPGVGVP
GVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGEGVPGEGVPGVGVPGMGVPGVGVPGVGVPGVGVPG
VGVPGVGVPGVGVPGVGVPGEGVPGEGVPGVGVPGMGVPGVGVPGVGVPGVGVPGVGVPGVGVPGV
GVPGVGVPGEGVPGEGVPGWGSDGVVVEIGKVTGSVGTTVEIPVYFRGVPSKGIANCDFVFRYDPNVLEII
GIDPGDIIVDPNPTKSFDTAIYPDRKIIVFLFAEDSGTGAYAITKDGVFAKIRATVKSSAPGYITFDEVGGFADN
DLVEQKVSFIDGGVNVGNAT

iLOV-Doc (green: iLOV, pink: Dockerin)

MGTDSLEFIASKLALEVLFQGPLQHHHHHHPWTSASGSPEFIEKNFVITDPRLPDNPIIFASDGFLELTEYSR
EEILGRNARFLQGPETDQATVQKIRDAIRDQRETTVQLINYTKSGKKFWNLLHLQPVRDQKGELQYFIGVQL
DGSDHVELKLPRSRKVVPGTPSTKLYGDVNDDGKVNSTDAVALKRYVLRSGISINTDNADLNEDGRVNST
DLGILKRYILKEIDTLPYKN

GFP-Doc (green: GFP, pink: Dockerin)

MGTDSLEFIASKLALEVLFQGPLQHHHHHHPWTSASSGGEELFAGIVPVLIELDGDVHGHKFSVRGEGEGD
ADYGKLEIKFICTTGKLPVPWPTLVTTLCYGIQCFARYPEHMKMNDFFKSAMPEGYIQERTIQFQDDGKYKT
RGEVKFEGDTLVNRIELKGKDFKEDGNILGHKLEYSFNSHNVYIRPDKANNGLEANFKTRHNIEGGGVQLA
DHYQTNVPLGDGPVLIPINHYLSTQTKISKDRNEARDHMVLLESFSACCHTHGMDELYRKVVPGTPSTKLY
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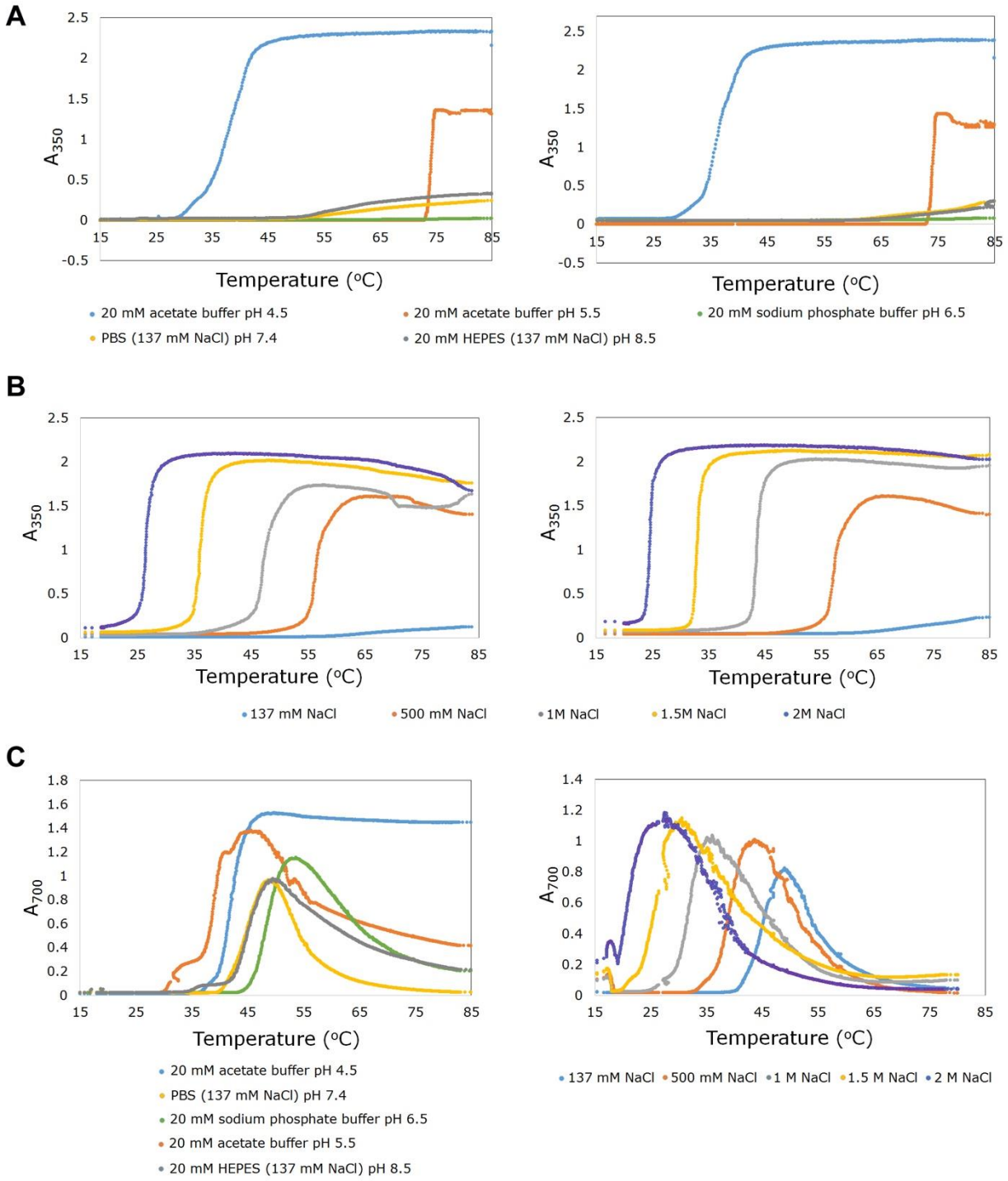


Figure S1. Phase transition curves showing the responsive characteristics of the ELP-Coh(s) upon changes of **(A)** pH change (left: $(M_1V_7E_2)_9$-Coh, right: $(M_1V_7E_2)_{12}$-Coh) and **(B)** NaCl concentration, as well as **(C)** that of the pNIPAm-mNPs.

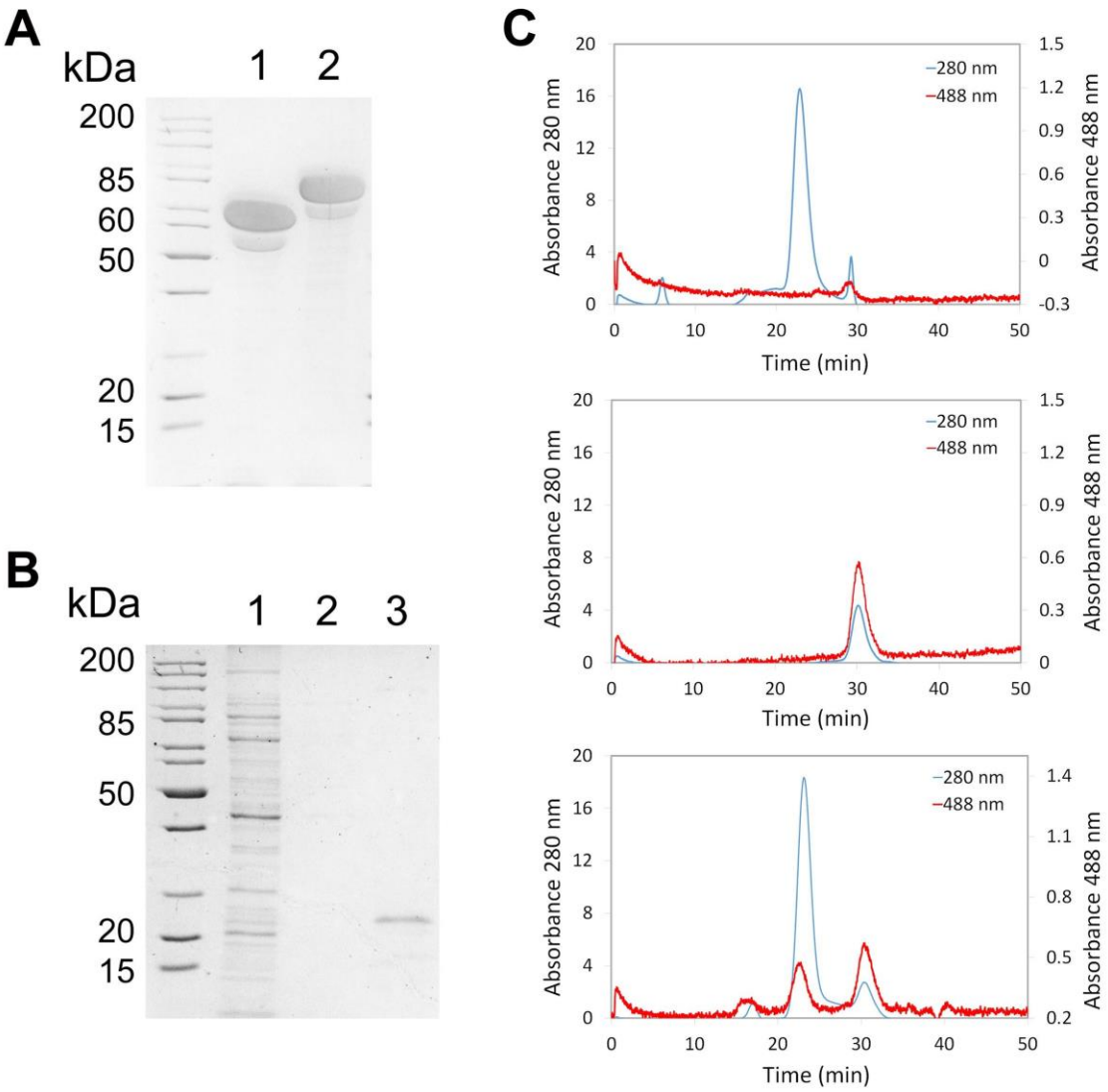


Figure S2. SDS-PAGE analysis of purified **(A)** ELP-Coh(s) (lane 1: $(M_1V_7E_2)_9$-Coh, lane 2: $(M_1V_7E_2)_{912}$-Coh) and **(B)** iLOV-Doc (lane 1: flowthrough, lane 2: 25 mM imidazole-wash fraction, lane 3: 500 mM imidazole eluted fraction). **(C)** SEC profile of $(M_1V_7E_2)_9$-Coh (top), iLOV-Doc (middle) and mixture of the two proteins (bottom), showing interaction of the two proteins with the shift of the iLOV-Doc peak toward the ELP-Coh peak. Fluorescence of iLOV was detected with an excitation wavelength of 488 nm.

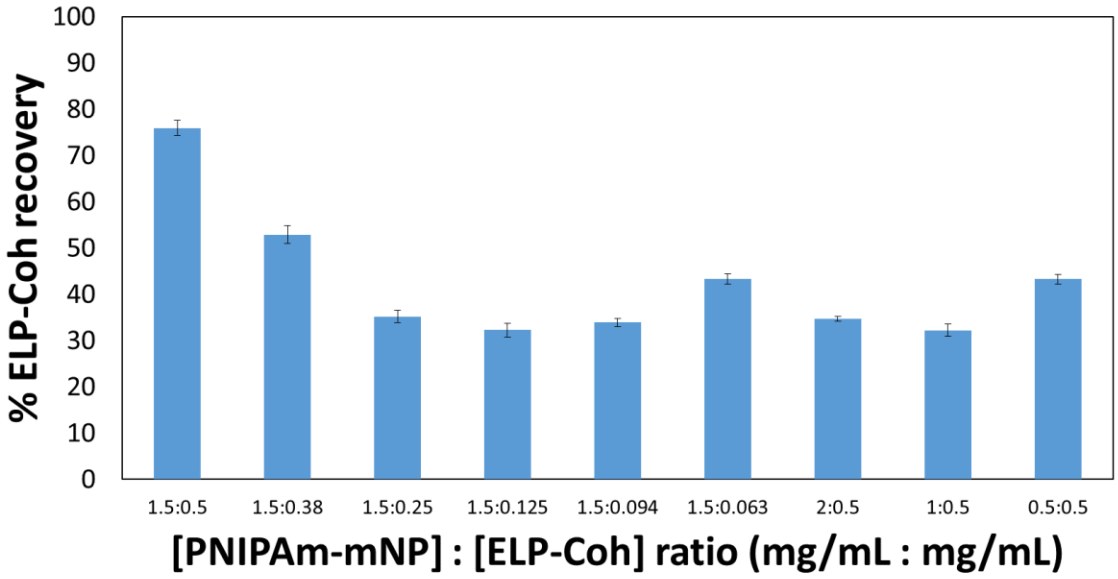


Figure S3. Recovery of the ELP-Coh ($(M_1V_7E_2)_9$) in fraction Sup2 after its co-aggregation with the pNIPAm-mNPs when using different concentration ratios of the ELP-Coh and the pNIPAm-mNPs.

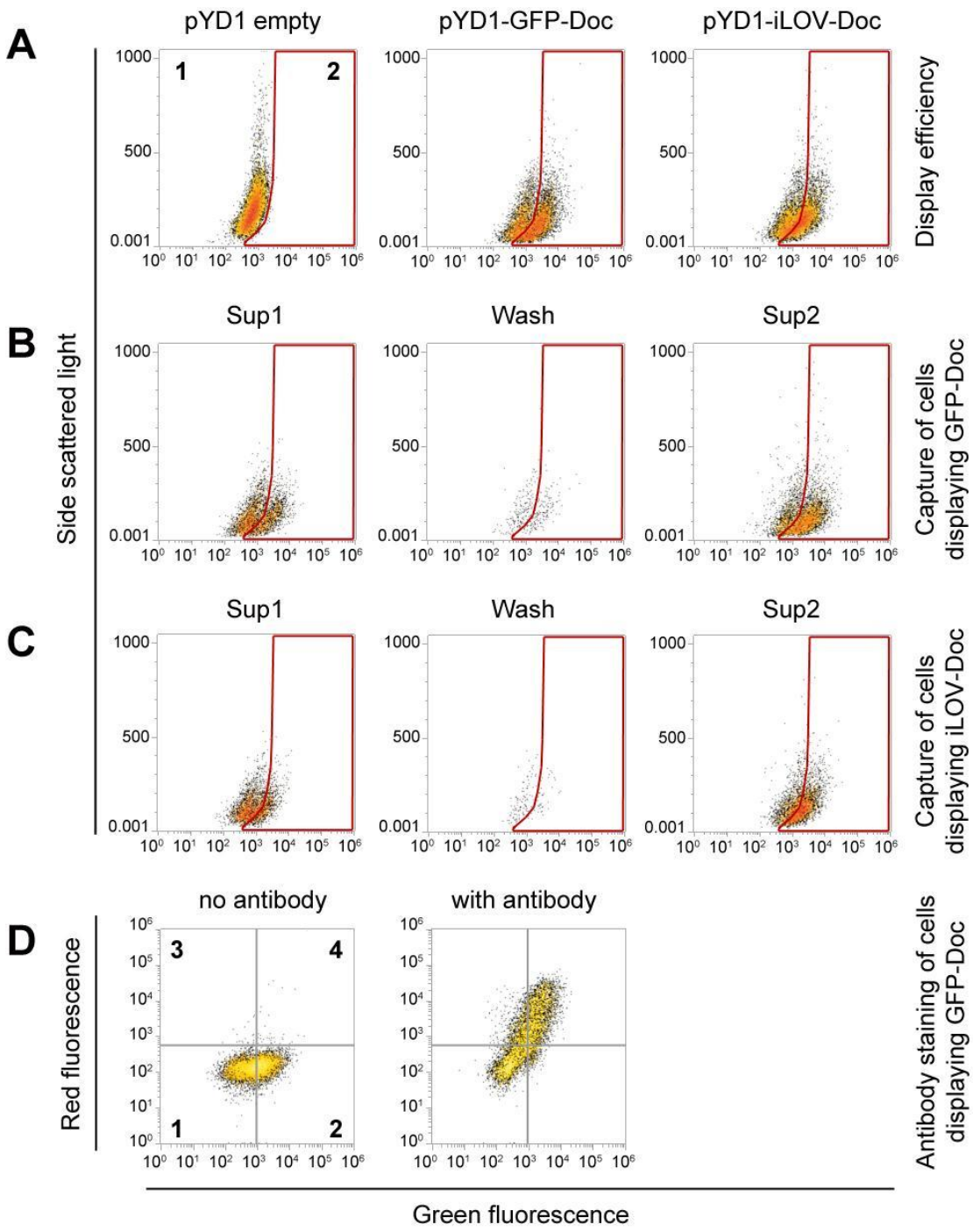


Figure S4. Flow cytometric result. **(A)** From left to right: cells carrying empty pYD1 vector, pYD1-GFP-Doc and pYD1-iLOV-Doc after 40hr-induction at 20°C. **(B)** Cell fractions from capture experiment of **(B)** GFP-Doc or **(C)** iLOV-Doc displaying cells using the pNIPAm-mNPs:ELP-Coh system. From left to right: Unbound (Sup1), Wash and released (Sup2). The gate (number 2, marked in red) was used to determine cells positive for green fluorescence. **(D)** Antibody staining of GFP-Doc displaying cell population mentioned in (A). The gates to determine negative cells for both fluorescence, negative for red fluorescence (Alexa 555) only, negative for green fluorescence (GFP), and positive for both fluorescence are marked with number 1, 2, 3, and 4, respectively.

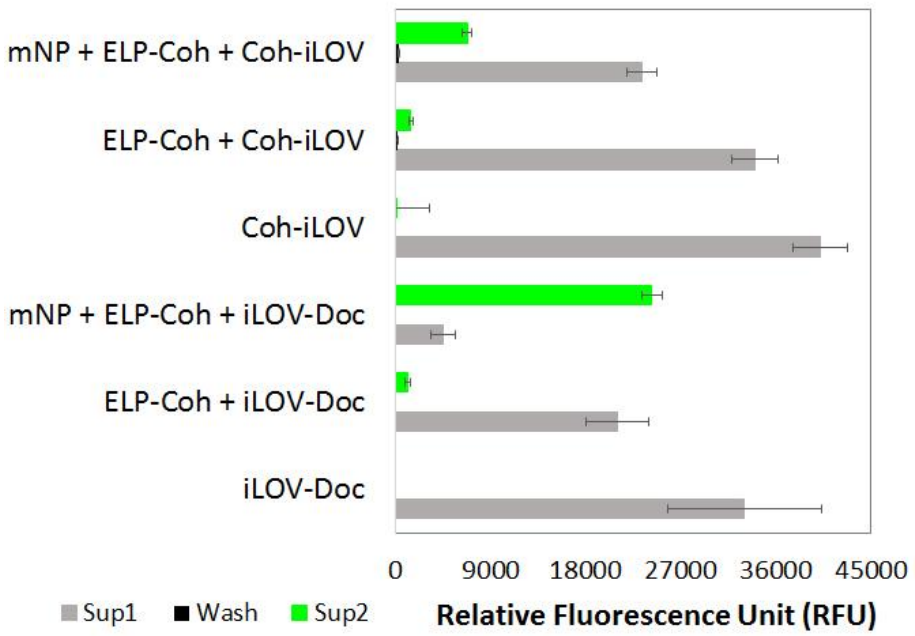


Figure S5. Fluorescence of the non-captured fraction (Sup1), wash fraction (Wash) and released fraction (Sup2) (with standard error bars display) under different treatment conditions. The Coh-iLOV was used as a non-binding negative control.

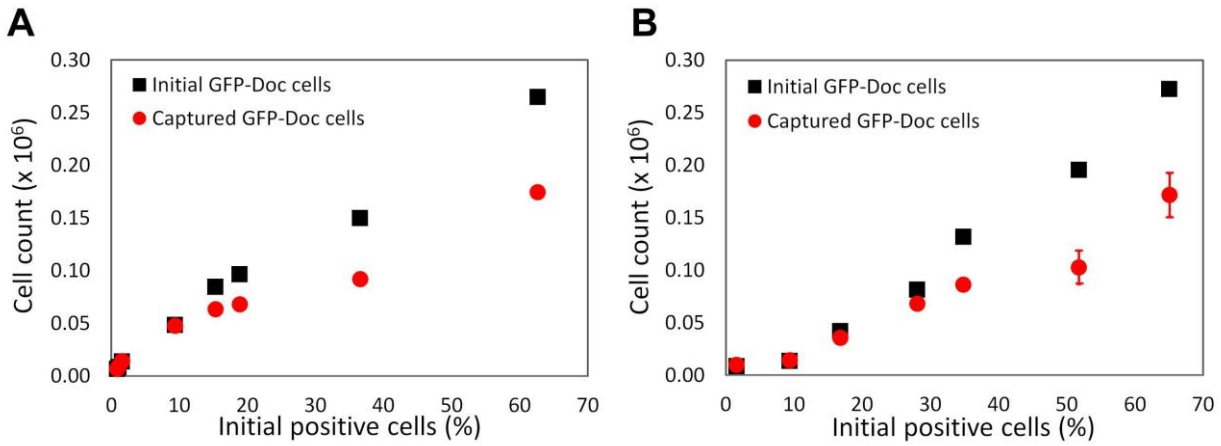
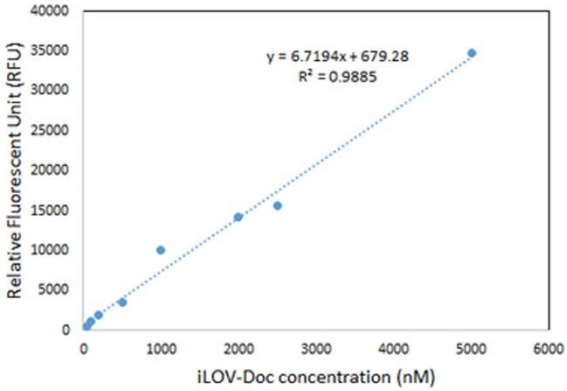
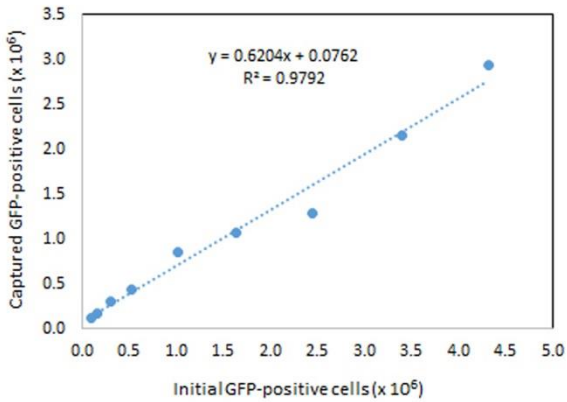


Figure S6. Comparison of number of GFP-positive cells captured by using the pNIPAm-mNPs:ELP-Coh system with the number of GFP-positive cells in the starting sample prepared in **(A)** TBS-Ca buffer and **(B)** TBS-Ca supplemented with 10% human blood plasma.



		standard error
slope	6.72	0.30
intercept	679.28	633.66
Detection limit	311.20 nM	



		standard error
slope	3302.12	160.17
intercept	4872.83	5150.30
Detection limit	0.38 x 10^6 cells/mL	

Figure S7. Standard curve and detection limit for detecting **(A)** iLOV-Doc and **(B)** yeast cells displaying GFP-Doc in TBS + 10% human blood plasma using the pNIPAm-mNP:ELP-Coh.